EXECUTIVE SUMMARY

The purpose of performance standards is to communicate the basis by which validated new proprietary (e.g., copyrighted, trademarked, registered) and nonproprietary test methods have been determined to have sufficient accuracy and reliability for specific testing purposes. Performance standards can then be used to evaluate the accuracy and reliability of other test methods that are based on similar scientific principles and that measure or predict the same biological or toxic effect. The three elements of performance standards are: 1) essential test method components (i.e., structural, functional, and procedural elements of a validated test method that a proposed, mechanistically and functionally similar test method should adhere to); 2) a minimum list of reference chemicals that is used to assess the accuracy and reliability of the proposed test method; and 3) the accuracy and reliability values that should be achieved by the proposed test method when evaluated using the minimum list of reference chemicals.

ICCVAM previously evaluated and recommended four validated test methods for assessing the dermal corrosivity hazard potential of chemicals: Corrositex®, EPISKIN™, EpiDerm™ (EPI-200), and the rat skin transcutaneous electrical resistance (TER) Assay. Subsequently, the EPA requested that ICCVAM establish performance standards for the three proprietary dermal corrosivity test methods (Corrositex®, EPISKIN™, EpiDerm™ [EPI-200]) and the non-proprietary rat skin TER test method. In response, the ICCVAM Dermal Corrosivity and Irritation Working Group (DCIWG) developed proposed performance standards based on these validated *in vitro* test methods. In a *Federal Register* Notice published on July 1, 2003**,** NICEATM invited public comment on the proposed performance standards for the three types of validated *in vitro* test methods for assessing dermal corrosivity hazard potential of chemicals. Comments on the draft document were also obtained during a public meeting of the NICEATM/ICCVAM Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) in August 2003, and the EPA Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) in October 2003. All comments were considered by the DCIWG and ICCVAM during development of this final document.

This document describes the performance standards that should be met by *in vitro* corrosivity test methods that utilize membrane barrier test systems, cultured human skin model systems, or the rat skin TER test method. These three types of *in vitro* corrosivity test methods have been recommended by ICCVAM as screening assays to identify corrosive substances based on data from the respective validated reference test method. The extent to which proposed test methods that are mechanistically and functionally similar to the validated reference test methods must demonstrate comparable performance should be considered on a case-by-case basis. While it would be desirable for such test methods to have reliability and accuracy values at least as good as that of the corresponding validated reference test method, some flexibility might be acceptable to the extent that it would not compromise the ultimate protection of human and animal health. For example, a test method with lower specificity will have a higher false positive rate, which may be undesirable because this results in erroneous classification into a more hazardous category, but does not result in lowered protection of human health. A test method that has lower sensitivity will result in higher false negative rates. However, because these test methods are recommended as screening tests, this will simply result in a greater number of positive corrosivity test results in the first animal tested for dermal irritancy. For future test methods proposed as replacements

for existing test methods, minimum acceptable false positive and false negative rates will likely be recommended by ICCVAM, based on what is necessary to provide for an equivalent or better protection of human and animal health or the environment.

In Vitro Membrane Barrier Test Systems for Skin Corrosion

Validation studies have been completed for an *in vitro* membrane barrier test system commercially available as Corrositex®. Based on its scientific validity, this test method has been recommended for use as part of a tiered testing strategy for assessing the dermal corrosion hazard potential of chemicals, whereby any substance that qualifies for testing can be evaluated. In addition, this test method may be used to make decisions on the corrosivity and noncorrosivity of specific classes of chemicals (e.g., organic and inorganic acids, acid derivatives[1], and bases) for certain transport testing circumstances. The basis of this test system is that it detects membrane damage caused by corrosive test substances. The test substance is first evaluated to determine if it is compatible with the test procedure. If compatible, the substance is evaluated for category of acid or base (strong or weak) to determine the appropriate time scale to use to classify the potential corrosivity of the test substance. Finally, a compatible substance is applied to the surface of the artificial membrane barrier. The time it takes for the test substance to penetrate through the membrane barrier to an underlying indicator solution determines the corrosivity classification of that test substance. Penetration of the barrier might be measured by a number of procedures, including a color change in a pH indicator dye or other properties of the solution below the barrier (e.g., electrical conductivity).

Investigators using *in vitro* membrane barrier test systems for skin corrosion must be able to demonstrate that the assay is valid for its intended use. This includes demonstrating that different preparations are consistent in barrier properties, capable of maintaining a barrier to noncorrosive substances, and able to categorize the corrosive properties of chemicals across the various subcategories of corrosivity described by the United Nations (UN) Packing Group classification system. A sample protocol for the validated reference test method is available at *http://iccvam.niehs.nih.gov*.

Essential test method components have been developed for *in vitro* membrane barrier test systems for corrosivity. These include the physical components of the test method (e.g., membrane barrier, categorization solutions, indicator solution); a test substance categorization system; processes for determining test substance compatibility and test substance categorization; procedures for assembly of the physical components of the test method; proocedures for application of a test substance; appropriate control substances (solvent controls, positive [corrosive] controls, negative [noncorrosive] controls, benchmark controls); procedures to measure membrane barrier penetration; interpretation of results; classification of test substances with regard to corrosivity potential; and elements of the test report. The test report provides the following information: test and control substances, justification of the test method and protocol used, test method integrity, criteria for an acceptable test, test conditions, results, description of other effects observed, discussion of the results, and conclusion.

[1] "Acid derivative" is a non-specific class designation and is broadly defined as an acid produced from a chemical substance either directly or by modification or partial substitution. This class includes anhydrides, haloacids, salts, and other types of chemicals.

ICCVAM recommends the use of a minimum list of 40 reference chemicals to evaluate the reliability and the accuracy of test methods similar to Corrositex®. The distribution of chemicals in this list by corrosivity and UN Packing Group classification are 12 noncorrosive chemicals and 28 corrosive chemicals (9 UN Packing Group I, 9 UN Packing Group II, 10 UN Packing Group III). When evaluated using this minimum list of recommended reference chemicals, the reliability and accuracy of the proposed *in vitro* membrane test method should be, at a minimum, comparable to that of the validated reference test method. ICCVAM also recommends that 12 of the reference chemicals (3 noncorrosives and 3 in each UN Packing Group classification) be used by laboratories to evaluate their proficiency in the appropriate use of Corrositex®.

In Vitro Human Skin Model Systems for Skin Corrosion

Pre-validation and validation studies have been completed for an *in vitro* human skin cell culture model system commercially available as EPISKIN™. Based on its scientific validity, this test method has been recommended for the testing of all classes of chemicals and for inclusion in tiered testing strategies as part of a tiered or weight-of-evidence evaluation. In addition to EPISKIN™, a related human skin cell culture model corrosivity test method marketed as EpiDerm™ (EPI-200) has been validated and recommended for the same use as EPISKIN™. Neither test method has been validated for categorizing the corrosive properties of chemicals across the three UN Packing Group subcategories of corrosivity.

The test material is applied topically to a three-dimensional human keratinocyte culture model, comprised of at least a reconstructed epidermis with a functional stratum corneum. Corrosive substances are identified by their ability to induce a decrease in cell viability below defined threshold levels at specified exposure periods. The principle of the human skin model assay is based on the premise that corrosive chemicals are able to penetrate the stratum corneum by diffusion or erosion, and are cytotoxic to the keratinocytes in the underlying layers. The use of test systems that include human-derived cells or tissue should be in accordance with applicable national and international laws, regulations, and policies.

Investigators using an *in vitro* human skin cell culture model system for skin corrosion must be able to demonstrate that the assay is valid for its intended use. This includes demonstrating that different preparations are consistent in barrier properties (i.e., capable of maintaining a barrier to noncorrosive substances, able to respond appropriately to weak and strong corrosive substances) and/or that any modification to the existing validated reference test method does not adversely affect its performance characteristics.

Essential test method components have been developed for *in vitro* human skin model test methods for skin corrosivity, based on Organisation for Economic Co-operation and Development (OECD) Test Guideline 431. The components are essentially the same as those described for Corrositex® with the additional inclusion of components related to *in vitro* human skin model systems. Human skin models can be obtained commercially (e.g., EPISKIN™, EpiDerm™ [EPI-200]) or they can be developed or constructed in the testing laboratory.

ICCVAM recommends the use of a minimum list of 24 reference chemicals (12 noncorrosives, 12 corrosives) to evaluate the reliability and accuracy of test methods similar to EPISKIN™.

When evaluated using this minimum list of recommended reference chemicals, the reliability and accuracy of the proposed *in vitro* membrane test method should be, at a minimum, comparable to that of the validated reference test method. ICCVAM also recommends that 12 of the reference chemicals (6 noncorrosives and 6 corrosives varying in corrosive potency) be used by laboratories to evaluate their proficiency in the appropriate use of Corrositex®.

In Vitro Skin Transcutaneous Electrical Resistance (TER) Tests for Skin Corrosion

Prevalidation and validation studies have been completed for the rat skin TER assay. Based on its scientific validity, this test method has been recommended for the testing of all classes of chemicals and for inclusion in tiered testing strategies as part of a tiered or weight-of-evidence evaluation.

The test substance is applied for up to 24 hours to the epidermal surface of skin discs in a two-compartment test system in which the skin discs function as the separation between the compartments. The skin discs are prepared from humanely killed 28 to 30 day-old rats. Corrosive substances are identified by their ability to produce a loss of normal stratum corneum integrity and barrier function, which is measured as a reduction in the TER below a specified level. For rat skin TER, a cutoff value of 5 kΩ has been selected based on extensive data for a wide range of substances where the majority of values were either clearly well above or well below this value. Generally, substances that are noncorrosive but irritating in animals do not reduce the TER below this cutoff value. However, the use of other skin preparations or other equipment to measure resistance may necessitate the use of a different cutoff value. In such situations, more extensive validation would be required. A dye-binding step is incorporated into the test procedure to confirm positive results. The dye-binding step determines if the increase in ionic permeability is due to physical destruction of the stratum corneum.

Investigators using an *in vitro* skin TER corrosivity test must be able to demonstrate that the assay is valid for its intended use. This includes demonstrating that different preparations are consistent in barrier properties (i.e., capable of maintaining a barrier to noncorrosive substances, able to respond appropriately to weak and strong corrosive substances) and/or that any modification to the existing validated reference test method does not adversely affect its performance characteristics.

Essential test method components have been developed for *in vitro* skin TER test methods for skin corrosivity, based on OECD Test Guideline 430. The components are essentially the same as those described for Corrositex® except that this test method includes essential components related to the use of animals, the physical measurement of transcutaneous electrical resistance, and dye binding procedures.

ICCVAM recommends that laboratories use a minimum list of 12 calibration chemicals (6 noncorrosives and 6 corrosives varying in corrosive potency) to evaluate their proficiency and to determine that the *in vitro* rat skin TER test method is performing as expected. A minimum list of 24 reference chemicals is recommended to determine if the reliability and accuracy of a new or modified *in vitro* skin TER test for skin corrosion is comparable to that of the validated reference test method. When a modified TER test method is evaluated using this minimum list of recommended reference chemicals, the reliability and accuracy of the proposed *in vitro* test method should be, at a minimum, comparable to that of the validated reference test method.